

What Is Claimed Is:

- 5 *Sub C*
1. A *Tma* DNA polymerase mutant which is modified at least one way selected from the group consisting of
 - (a) to reduce or eliminate the 3'→5' exonuclease activity of the polymerase;
 - (b) to reduce or eliminate the 5'→3' exonuclease activity of the polymerase; and
 - (c) to reduce or eliminate discriminatory behavior against a dideoxynucleotide.
 - 10 2. The DNA polymerase mutant of claim 1, which is modified at least two ways.
 3. The DNA polymerase mutant of claim 1, which is modified three ways.
 - 15 4. The *Tma* DNA polymerase mutant of claim 1 which comprises a mutation in the O-helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides.
 - 20 *B* *Sub D2* 5. The DNA polymerase of claim 4, wherein said O-helix is defined as RXXXKXXXFXXXYY, wherein X is any amino acid. *(Said ID NO. 1)*
 6. The *Tma* DNA polymerase as claimed in claim 5, wherein said mutation is a Phe⁷³⁰→Tyr⁷³⁰ substitution.
 7. The *Tma* DNA polymerase of claim 1, wherein said DNA polymerase is a *Tma* DNA polymerase having substantially reduced 3'→5' exonuclease activity.

8. The mutant *Tma* DNA polymerase as claimed in claim 7, wherein said mutant is a Asp³²³→Ala³²³ substitution.

Sub
5 E 2
9. ~~The mutant *Tma* DNA polymerase as claimed in claim 1, wherein said mutant polymerase comprises both a Phe⁷³⁰→Tyr⁷³⁰ substitution and a Asp³²³→Ala³²³ substitution.~~

10. The mutant DNA polymerase mutant of claim 1, wherein said DNA polymerase is a *Tma* DNA polymerase having substantially reduced 5'→3' exonuclease activity.

10 11. The mutant *Tma* DNA polymerase as claimed in claim 10, wherein said mutant polymerase has a deletion mutation in the N- terminal 5'→3' exonuclease domain.

12. The mutant *Tma* DNA polymerase as claimed in claim 11, wherein said mutant polymerase is devoid of the 219 N-terminal amino acids.

15 13. A vector comprising a gene encoding the DNA polymerase of claim 1.

14. The vector of claim 13, wherein said gene is operably linked to a promoter.

20 15. The vector of claim 14, wherein said promoter is selected from the group consisting of a λ -P_L promoter, a *tac* promoter, a *trp* promoter, and a *trc* promoter.

16. A host cell comprising the vector of claim 13.

17. A method of producing a DNA polymerase, said method comprising:

- (a) culturing the host cell of claim 16;
- (b) expressing said gene; and
- (c) isolating said DNA polymerase from said host cell.

18. The method of claim 17, wherein said host cell is *E. coli*.

19. A method of synthesizing a double-stranded DNA molecule comprising:

- (a) hybridizing a primer to a first DNA molecule; and
- (b) incubating said DNA molecule of step (a) in the presence of one or more deoxy- or dideoxyribonucleoside triphosphates and the DNA polymerase of claim 1, under conditions sufficient to synthesize a second DNA molecule complementary to all or a portion of said first DNA molecule.

20. The method of claim 19, wherein said deoxy- or dideoxyribonucleoside triphosphates are selected from the group consisting of dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-dGTP, dUTP, ddATP, ddCTP, ddGTP, ddITP, ddTTP, [α -S]dATP, [α -S]dTTP, [α -S]dGTP, and [α -S]dCTP.

21. The method of claim 19, wherein one or more of said deoxy- or dideoxyribonucleoside triphosphates are detectably labeled.

- (a) A method of sequencing a DNA molecule, comprising:
 - (a) hybridizing a primer to a first DNA molecule;
 - (b) contacting said DNA molecule of step (a) with deoxyribonucleoside triphosphates, the DNA polymerase of claim 1, and a terminator nucleotide;

(c) incubating the mixture of step (b) under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 5' termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.

22. The method of claim 22, wherein said deoxyribonucleoside triphosphates are selected from the group consisting of dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-dGTP, dUTP, [α -S]dATP, [α -S]dTTP, [α -S]dGTP, and [α -S]dCTP.

23. The method of claim 22, wherein said terminator nucleotide is ddTTP, ddATP, ddGTP or ddCTP.

24. The method of claim 22, wherein one or more of said deoxyribonucleoside triphosphates is detectably labeled.

25. The method of claim 22, wherein one or more of said terminator nucleotides is detectably labeled.

26. A method for amplifying a double stranded DNA molecule, comprising:

(a) providing a first and second primer, wherein said first primer is complementary to a sequence at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence at or near the 3'-termini of the second strand of said DNA molecule;

(b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of the DNA polymerase of claim 1, under conditions such that a third DNA molecule complementary to said first strand and a fourth DNA molecule complementary to said second strand are synthesized;

(c) denaturing said first and third strand, and said second and fourth strands; and

(d) repeating steps (a) to (c) one or more times.

27. The method of claim 27, wherein said deoxyribonucleoside triphosphates are selected from the group consisting of dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-dGTP, dUTP, $[\alpha\text{-S}]$ dATP, $[\alpha\text{-S}]$ dTTP, $[\alpha\text{-S}]$ dGTP, and $[\alpha\text{-S}]$ dCTP.

28. A kit for sequencing a DNA molecule, comprising:

(a) a first container means comprising the DNA polymerase of claim 1;

(b) a second container means comprising one or more dideoxyribonucleoside triphosphates; and

(c) a third container means comprising one or more deoxyribonucleoside triphosphates.

29. A kit for amplifying a DNA molecule, comprising:

(a) a first container means comprising the DNA polymerase of claim 1; and

(b) a second container means comprising one or more deoxyribonucleoside triphosphates.

30. A mutant *Tma* DNA polymerase having substantially reduced or eliminated 5'-3' exonuclease activity, wherein at least one of the amino acids

corresponding to Asp⁸, Glu¹¹², Asp¹¹⁴, Asp¹¹⁵, Asp¹³⁷, Asp¹³⁹, Gly¹⁰², Gly¹⁸⁷, or Gly¹⁹⁵ corresponding to *Tne* DNA polymerase has been mutated.

31. A vector coding for the mutant DNA polymerase of claim 31.

32. A host cell comprising the vector of claim 32.

33. A method of producing a mutant *Tma* DNA polymerase having substantially reduced or eliminated 5'-3' exonuclease activity, wherein at least one of the amino acids corresponding to Asp⁸, Glu¹¹², Asp¹¹⁴, Asp¹¹⁵, Asp¹³⁷, Asp¹³⁹, Gly¹⁰², Gly¹⁸⁷, or Gly¹⁹⁵ corresponding to *Tne* DNA polymerase has been mutated, comprising

- (a) culturing the host cell of claim 33;
- (b) expressing the mutant DNA polymerase; and
- (c) isolating said mutant DNA polymerase.

34. A method of preparing cDNA from mRNA, comprising

- (a) contacting mRNA with an oligo(dT) primer or other complementary primer to form a hybrid, and
- (b) contacting said hybrid formed in step (a) with the *Tne* DNA polymerase or mutant of claim 1 and dATP, dCTP, dGTP and dTTP, whereby a cDNA-RNA hybrid is obtained.

35. A method of preparing dsDNA from mRNA, comprising

- (a) contacting mRNA with an oligo(dT) primer or other complementary primer to form a hybrid, and
- (b) contacting said hybrid formed in step (a) with the *Tne* DNA polymerase or mutant of claim 1, dATP, dCTP, dGTP and dTTP, and an oligonucleotide or primer which is complementary to the first strand cDNA; whereby dsDNA is obtained.

add A2 7 add